crosslinkers preserved. It is interesting to note that the dissipative and plastic responses of such networks to applied loads are more similar to crack propagation in solids than to standard polymer rheology, where standard mechanisms for energy dissipation are hydrodynamics, filament contour fluctuations, etc. Our results will have important implications for understanding mechanical properties of cytoskeletons, where networks of MTs and Factin bundles crosslinked by different flexible and transient crosslinks are locally deformed by transport of intracellular cargos and by the large-scale structural changes in cell division, motility and morphogenesis.

2828-Plat
Asymmetric Force Response Reveals Mechanical Role in Spindle Protein Localization
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The mitotic spindle is the self-organized, microtubule based structure which mechanically segregates chromosomes during cell division. The spindle 'parts-list' includes microtubules, motor proteins, and non-motor microtubule-associated proteins (or MAPs), and the biochemical properties of many of these components have been well studied. By comparison, our understanding of the force-dependent behavior of many key interactions remains limited. In particular, we do not understand the role that cross-linking MAPs play in providing mechanical stability within the highly dynamic spindle, or how force regulates the function and localization of these proteins. To address this shortcoming, we examine the force-dependent response of NuMA, the major cross-linking MAP of minus-end focused parallel microtubules at the spindle pole. Combining data taken with single molecule TIRF-based imaging and optical trapping methods, we show that NuMA/microtubule interactions generate resistive, friction-like forces which approach ~1pN when dragged at velocities in the micron/sec range. Unexpectedly, the mechanical response is asymmetric, with NuMA sliding more easily towards the minus ends of microtubules than the plus ends. For comparison, we show that PRC1, a dimeric protein which cross-links antiparallel microtubules at the spindle midzone in anaphase, does not possess such an asymmetric behavior under force. We further perform computer simulations on parallel microtubules cross-linked by 'dimerized' NuMA (effectively a minimal structural unit of the spindle pole), and show that in the presence of small oscillatory perturbations, NuMA will migrate to the minus ends. These combined results suggests a mechanism for autonomous localization to the spindle poles, and may reveal a possible mechanical principle underlying spindle self-organization.

2829-Plat
Zipping Dynamics and Turgor Pressure during Dorsal Closure in Early *Drosophila* Development
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Biomechanical processes engaged in morphogenesis require forces to shape multiple tissues in a three-dimensional pattern during metazoan development. Dorsal closure, an essential stage of *Drosophila* embryogenesis, serves as an *in-vivo* model system for cell sheet movements during development and wound healing. During closure two flanks of lateral epidermis approach to close an eye-shaped gap that is initially occupied by a transient amnioserosa tissue in the dorsal opening. Based on a two-dimensional approximation, the time-dependent geometry of the dorsal opening previously has been quantified by four biomechanical processes collectively including apical constriction of amnioserosa cells, tension due to an actomyosin-rich purse string within each leading edge, adhesive zipping at each corner (canthus) of the eye-shaped opening, and resistance due to the lateral epidermis[1]. To more fully understand dorsal closure, we have moved beyond the two-dimensional approximation and report here our three-dimensional investigation. We investigated embryos with GFP/RFP labeled DE-cadherin, myosin, and/or moesin (actin) using time-lapsed confocal microscopy. We observed zipping to be an unexpectedly and remarkably three-dimensional process. The amnioserosa was pushed below the two leading edges of lateral epidermis as they zipped at each canthus. Just prior to zipping, the leading edges slid over the amnioserosa towards the anteroposterior axis. In addition, during early-to-mid stages of closure we observed the amnioserosa in the geometry of a dome. Segmenting this asymmetric dome and fitting with Laplace's formula quantified the turgor pressure. Furthermore, the purse strings that define the dorsal opening were curved in three dimensions with significant bends towards the embryo interior near each canthus. This research has been supported by the NIH, grant No. 33830.
1. *Science* 300:145-149 (2003).

Platform: Systems Biophysics

2830-Plat
A Model of Nuclear Organization Demonstrates the Effect of Nuclear Envelope - Chromosome Contacts on 3D Organization of Chromosomes
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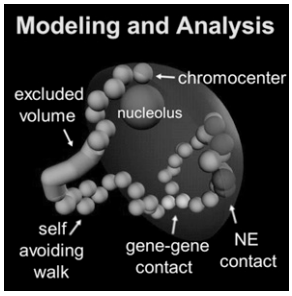
We describe a method for modeling organization of the interphase nucleus, and its application to polytene chromosomes of Drosophila salivary glands. The model represents chromosomes as polymer chains confined within the nucleus. Physical parameters of the model are taken directly from experiment, no fitting parameters are introduced. The model is used to simulate chromosome tracing experiments. When applied to previously published data 33 new chromosome_-nuclear envelope (Chr-NE) contacts are revealed. Most of these new Chr-NE contacts correspond to intercalary heterochromatin - gene poor, dark staining, late replicating regions of the genome; only three correspond to euchromatin - gene rich, light staining, early replicating regions of the genome. Analysis of regions least likely to form Chr-NE contacts reveals that these are mostly euchromatic, but may contain late replication regions or intercalary heterochromatin. We show that Chr-NE contacts may affect long range gene-gene interactions: depending on the chromosome contour length between two contacts, gene-gene interaction probability may increase or decrease. We also develop methods to objectively quantify chromosome territories and intertwining and discuss the corresponding experimental observations.



2831-Plat
Effects of Fluctuation of Chromosome Conformation and Spatial Arrangement of Genes on the Pattern of Gene Expression
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Recently, conformation of all chromosomes in the interphase nucleus of budding yeast has been inferred from the Chromosome Conformation Capture-on-Chip (4C) data (Duan et al., Nature (2010)). However, it has not yet been ascertained how the conformational fluctuation around their mean structures affects the regulation of gene expression. To clarify this issue, we developed a dynamical structural model of interphase chromosomes in budding yeast (Tokuda et al., Biophys. J. (2012)). In the present paper, the effects of the conformational fluctuation and the arrangement of genes on the pattern of gene expression are discussed by using this coarse-grained chromosome model. In particular, the observed difference in the pattern of gene expression between the *yku70 esc1* mutant which abrogates telomere anchoring and the wild-type strain (Taddei et al., Genome Res. (2009)) is studied. In the data of Taddei et al., 32 genes are expressed at higher levels and 28 genes are expressed at lower levels in the *yku70 esc1* mutant than in the wild-type strain. We examine the reason of this misregulation by comparing the fluctuation of the chromosome conformation and spatial arrangement of genes in the case that telomeres are not anchored to the nuclear periphery with that in the anchored case.

2832-Plat
Modeling Stochastic Gene Expression in Growing Cells
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Gene expression is an inherently noisy process. Fluctuations arise at many points in the expression of a gene, as all the salient reactions such as transcription, translation, mRNA degradation etc. are stochastic processes. The flucatuations become important when the cellular copy numbers of the relevant molecules (mRNA or proteins) are low. We investigate different sources of noise in gene expression by considering several models in which protein synthesis and partitioning of proteins during cell dividision are described in either a stochastic or a deterministic way. For regulated genes, a computational complication arises from the fact that protein synthesis rates depend on the concentrations of the transcription factors that regulate the corresponding genes. Because of the growing cell volume, such rates are effectively time-dependent. We deal with the effects of volume growth compuationally using a rather simple method: the growth of the cell volume is incorporated in our simulations by stochastically adding small volume elements to the cell volume.

As an application of this method we study a gene circuit with positive autoregulation that exhibits bistability. We show how the region of bistability becomes diminshed by increasing the effect of noise via a reduced copy number of the regulatory protein. Cell volume determines the region of bistability for different noise strength. The method is general and can also be applied to other cases where synthesis of proteins are regulated and an appropriate analytical description is difficult to achieve.

2833-Plat
Spatio-Temporal Measurements and Modeling of Genetic Expression
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Single-molecule, single-cell studies of genetic expression have provided key insights into how cells respond to external stimuli [Munsky, B., et al., Science (2012)]. By directly measuring copy numbers of individual bio-molecules in cells, it is now possible to obtain statistical measures of the spatio-temporal distributions of key signaling and regulatory networks. Such comprehensive datasets can be used to infer system-level models that yield quantitative insight into cellular regulation, predict cellular responses in new experimental conditions, and suggest more revealing experiments to uncover regulatory dynamics. The integration of single-molecule spectroscopy, biochemistry, and numerical modeling is a powerful multi-disciplinary approach to investigating cellular response at the genetic level.
A key issue we seek to address is what types of fluctuations are most informative about the underlying gene regulatory process. In other words, how much experimental resources should be spent to measure (i) temporal, (ii) spatial, or (iii) cell-to-cell fluctuations? As an example, we studied Interluekin 1-alpha (IL1α) mRNA expression within human THP-1 cells during stimulus response to lipopolysaccharide (LPS). By spatially resolving individual mRNA using multiplexed single molecule FISH [Femino A.M., et al., Science (1998), Raj A., et al., Nat Meth (2008)] in large populations of single cells at multiple times points, we quantified all three fluctuation types.
We expanded the common bursting gene expression model [Peccoud, J., Theoretical Population Biology (1995)] and derived a set of linear ODEs to describe the mean, variance, and co-variance of nuclear and cytoplasmic IL1α mRNA. We fit this model to multiple single-cell datasets. Comparing models inferred from each data set, we are able to draw conclusions on which fluctuation types are most revealing about the underlying system's mechanisms and parameters, providing feedback for new experiments. The approach developed here is applicable to any eukaryotic gene expression pathway.

2834-Plat
Gene Location and DNA Density Determine Transcription Factor Distributions in E. Coli
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The diffusion coefficient of the canonical transcription factor Lac Repressor, LacI, within living Escherichia coli has been measured directly by in vivo tracking to be D = 0.4 μm^2/s. At this rate, simple models of diffusion lead to the expectation that LacI and other proteins will rapidly homogenize throughout the cell.
We have tested this expectation of spatial homogeneity by high-throughput single molecule visualization of LacI molecules non-specifically bound to DNA in fixed cells to generate an ensemble average of the steady-state distribution of protein in the cell. Contrary to expectation, we find that the distribution of LacI depends on the spatial location of its encoding gene. We demonstrate that the spatial distribution of LacI is also determined by the local state of DNA compaction, and that E. coli can dynamically redistribute proteins by modifying the state of its nucleoid. We then show that LacI inhomogeneity increases the strength with which targets located proximally to the LacI gene are regulated. Finally, we propose a model for intranucleoid diffusion which can reconcile these results with previous measurements of LacI diffusion.

2835-Plat
Transcriptional Control by Regulated Oligomerization
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Numerous transcription factors self-assemble into different order oligomeric species in a way that is actively regulated by the cell. The functional role of this widespread process is not yet completely understood. Here we capture the effects of regulated oligomerization in gene expression with a novel quantitative framework. We show that this mechanism provides precision and flexibility, two seemingly antagonistic properties, to the sensing of diverse cellular signals by systems that share common elements present in transcription factors like p53, NF-κB, STATs, Oct, and RXR. Applied to the nuclear hormone receptor RXR, this framework accurately reproduces a broad range of classical, previously unexplained, sets of gene expression data and corroborates the existence of a precise functional regime with flexible properties that can be controlled both at a genome-wide scale and at the individual promoter level.

2836-Plat
Novel Mechanism for Noise Reduction between Nuclear Transcriptional Activity and Cytoplasmic MRNA Distributions Living Fly Embryos
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Noise in gene expression has been shown to play an important role in various organisms. In particular, transcriptional bursts are thought to be a common mode for mRNA output. Yet, the final patterns of gene expression, especially in the case of multicellular organisms such as Drosophila melanogaster, are highly precise and reproducible. In order to address this apparent contradiction, we present a strategy to measure transcriptional dynamics in living fly embryos by monitoring the production of mRNA at their sites of transcription on DNA loci in real time simultaneously in hundreds of individual nuclei. In particular, we measure the transcriptional activity of the hunchback promoter as a function of the position along the embryo throughout the first three hours of development. We observe ''waves'' of transcription associated with the progressing mitotic cycles, where transcription rises steadily in the beginning of the nuclear cycle, peaks mid-way through it and disappears during mitosis. Although the overall shape of these transcription waves is similar, we see a high degree of variability among nuclei. However, we see no evidence of transcriptional bursting. Single molecule mRNA FISH reveals that this variability in transcription rate does not translate into noise in the cytoplasmic mRNA distribution suggesting the need for a mechanism of noise rectification. In fact, we observe an overall 4-fold noise reduction between nuclear activity and cytoplasmic mRNA levels. using a simple stochastic model we demonstrate that our noise measurements can only be explained through a combination of both temporal and spatial averaging.

2837-Plat
Shaping Gene Expression in Artificial Cellular Systems by Cell-Inspired Molecular Crowding
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Synthetic biology has made tremendous recent strides in constructing artificial cellular systems using minimal cell components *ex vivo*, creating an experimental platform for characterizing the behavior of isolated cellular modules and a form of biotechnology for the controlled operation of artificial cells. The robustness and efficiency of these systems are nonetheless challenging to control, in part because artificial cells establish an environment that is still very different from that of actual living cells. Here, we present a novel approach towards bridging the gap between artificial and true cell environments by developing artificial cells incorporating controlled macromolecular crowding, mimicking a key feature of natural cells known to dramatically influence biochemical kinetics. We demonstrate the value of our approach by showing that molecular crowding enhances gene expression and confers robustness against perturbations of gene environments. We further elucidate the underlying mechanisms of these phenomena at the single molecule level by demonstrating how large crowding molecules decrease diffusion of T7 RNA polymerase, but increase its binding to a T7 RNAP promoter. Based on single-molecule results, we further show that the impact of molecular crowding on gene circuits is enhanced by weak genetic components and maximized by a negative feedback loop. By bridging a key gap between artificial cell technology and the environment of living cells, we demonstrate the importance of intracellular crowding to efficient and robust function of biological circuits and suggest new engineering principles for controlled modulation of synthetic genetic systems.

Platform: Protein Assemblies, Aggregates, & Chaperones

2838-Plat
Biophysical Analysis of a Novel Drug Delivery Vector: ELP[V5G3A2-150]
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